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ARTICLE

Stored Human Urine Supplemented with Wood Ash as Fertilizer in Tomato (*Solanum lycopersicum*) Cultivation and Its Impacts on Fruit Yield and Quality

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This study evaluates the use of human urine and wood ash as fertilizers for tomato cultivation in a greenhouse. Tomatoes were cultivated in pots and treated with 135 kg of N/ha applied as mineral fertilizer, urine + ash, urine only, and control (no fertilization). The urine fertilized plants produced equal amounts of tomato fruits as mineral fertilized plants and 4.2 times more fruits than nonfertilized plants. The levels of lycopene were similar in tomato fruits from all fertilization treatments, but the amount of soluble sugars was lower and Cl^- was higher in urine + ash fertilized tomato fruits. The β-carotene content was greater and the NO_3^- content was lower in urine fertilized tomato fruits. No enteric indicator microorganisms were detected in any tomato fruits. The results suggest that urine with/without wood ash can be used as a substitute for mineral fertilizer to increase the yields of tomato without posing any microbial or chemical risks.

KEYWORDS: Glucose; lycopene; nitrogen; tomato; urine; wood ash; β-carotene

INTRODUCTION

Human urine and wood ash can be used as a nutrient resource in agriculture. Urine contains high amounts of N (nitrogen), P (phosphorus), and K^+ (potassium) (*1*), and wood ash contains high levels of P, K^+, Ca^{2+} (calcium), and Mg^{2+} (magnesium) (*2*, *3*). Thus, these elements present in urine and wood ash could be useful as fertilizers. In general, pure human urine contains very few enteric microorganisms if there is no fecal contamination (*4*).

Urine has been successfully used to fertilize cucumber (*4*), maize (*5*), cabbage (*6*), wheat (*7*), and tomato (*8*). In general, the amount of N in wood ash is low/none; therefore, ash could represent a good supplement with urine fertilizer. This would be especially beneficial in acidic soils. However, only a few scientific studies have investigated the use of wood ash in agriculture (*2*, *9*−*12*), and the combination of urine + ash as fertilizer has not previously been addressed. It is important to study the use of urine and wood ash as fertilizer with different crop plants because of their different nutrient demands and the nutrient contents in urine and wood ash. Furthermore, it is important to determine the bioavailability of different nutrients present in these fertilizers.

The main objectives of this study were to evaluate (1) the fertilizer value of pure urine and urine + wood ash mixture used in tomato cultivation, (2) the chemical and microbial quality of the tomato fruits with these fertilizer treatments, and (3) the taste of the tomato fruits fertilized in different ways. In this study, we hypothesized that the tomato yields and their microbial and chemical quality would be similar in mineral fertilized and urine + ash fertilized tomato fruits, somewhat higher compared to urine only and significantly higher compared to without fertilization. Tomato was selected for this study because it has a worldwide distribution, it is commonly cultivated in home gardens, and it is a common ingredient in many recipes.

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MATERIALS AND METHODS

Plant Materials and Cultivation. Tomato (*Solanum lycopersicum* L. Sparta F1) (Nelsons OY, Finland) were sown in pots at the research greenhouse of University of Kuopio (62.9° N and 27.7° E) on March 27, 2008. The growth conditions inside the greenhouse were day/night temperature 30/17 °C, light period 12/12 h, and humidity 100/26%. The fully grown seedlings were transplanted to larger pots (volume = 7.5 L, surface area = 491 cm^2, one plant per pot) on April 28, 2008. Five tables were used for placing the pots (10 per treatment); eight pots were placed on each table, that is, two pots from each fertilizer treatment. The experimental pots from four different treatments, namely, (1) no fertilizer (control), (2) mineral fertilizer, (3) urine + wood ash fertilizer, and (4) urine only fertilizer were placed in a zigzag formation on tables at similar illumination. The illumination was 5×10^4 lx at shoot top level and photosynthetically active radiation (PAR) ~700 μmol/m^2/s at top level. The two plants on the same table were calculated as one replicate, and the experiment was carried out with five replicates on five tables.

Plants were grown in a soil-containing mixture of peat and sand in ratio of 3:1 (**Table 1**). Before cultivation, the soil pH was very low (about pH 3) so the soil was limed (Dolomite lime Ca 30%) to all treatments in a similar manner. The cultivated soil was limed on April 1, 12, and 27 with 40 g/pot lime corresponding to 8.1 ton/ha lime. The soil pH reached about 5.6−6.6 after liming.

Fertilizer Treatments. Fertilization with mineral, urine + ash, and urine was estimated to provide a dose of 135 kg of N/ha, and the mineral and urine + ash treatments were also estimated to contribute 90 kg of P/ha (**Tables 2** and **3**). The mineral fertilizer (Puutarhan Y1, NPK 9−6−17 (6.5% NH_4^+-N and 2.5% NO_3^--N)), which also contained Mg 2%, S 10%, B 0.05%, Cu 0.1%, Fe 0.1%, Mn 0.7%, Mo 0.01%, Zn 0.1%, and

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Table 1. Chemical and Physical Parameters of Cultivated Soil before and after Fertilization and Cultivation[a]

		after					
parameter	before	none	mineral	urine + ash	urine	*F*	*P*
total-N (g)	0.25	1.5 ± 0.3 b	0.7 ± 0.3 a	1.2 ± 0.4 ab	0.9 ± 0.4ab	5.150	0.011
NO_3^--N (mg)	24.26	11.8 ± 2.4 b	16.5 ± 1.3 c	5.2 ± 0.5 a	9.1 ± 3.6 ab	22.334	0.0001
NO_2^--N (mg)	3.56	LDL	15.7 ± 16.7	6.1 ± 3.5	LDL		
NH_4^+-N (mg)	ND	5.9 ± 2.7 b	4.2 ± 0.8 ab	2.9 ± 0.7 a	2.5 ± 0.5 a	5.391	0.009
Cl^- (mg)	19.98	14.0 ± 2.8 a	12.9 ± 3.6 a	36.5 ± 9.9 b	49.7 ± 10.2 b	28.706	0.0001
P (mg)	340	411.1 ± 67.9 ab	315.3 ± 88.0 ab	429.5 ± 82.7 b	261.1 ± 55.1 a	4.698	0.015
K^+ (g)	1.6	1.3 ± 0.4 a	1.5 ± 0.2 a	1.7 ± 0.4 ab	2.0 ± 0.2 b	6.193	0.005
Ca^{2+} (g)	10.8	6.9 ± 1.4	6.5 ± 1.0	5.1 ± 1.3	5.9 ± 0.9		NS
Mg^{2+} (g)	6.3	4.5 ± 0.4 b	4.3 ± 0.5 ab	3.6 ± 0.6 a	3.6 ± 0.3 a	5.125	0.011
OM (%)	10.39	11.9 ± 2.1	11.1 ± 1.5	12.4 ± 3.0	12.7 ± 2.4		NS
pH	4.36	5.06	5.1	6.46	5.62		
conductivity (μs/cm)	13.5	67.1	144.7	68.7	54.0		

[a] The results are presented per kg of dry weight (DW) (Arithmetic mean (AM) ± Standard deviation (SD)) ($N = 5$). NS, not significant; ND, not determined; LDL, less than detection limits. Physical and chemical parameters from before and after cultivation of soil have not been statistically analyzed. The *P* and *F* values were from ANOVA analysis for comparison of different treatments only after cultivation.

Table 2. Chemical and Physical Parameters of the Urine and Wood Ash Used in This Study

nutrients and pH	urine (g/L)	wood ash (g/kg)
total-N[a]	8.36	ND
NO_3^--N	0.01	ND
NH_4^+-N	8.57	ND
total-P	0.7	36
PO_4^--P	2.03	ND
K^+	2	137
Ca^{2+}	ND	216
Mg^{2+}	ND	47
Cl^-	3.03	ND
pH	9.2	11.14

[a] Total-N contents in urine showed lower than NH_4^+-N, which might be due to loss of N during the burning process in the Kjehldal method of total-N analysis.

Table 3. Applied Amount of Nutrients and Fertilizers/Plant (491 cm^2) during the Entire Cultivation Period

nutrients and fertilizer	mineral fertilizer	urine + wood ash fertilizer	urine fertilizer
total-N (mg)	663	663 (663 + NK[a])	663
total-P (mg)	442	442 (57 + 385)	57
total-K (mg)	1253	1491 (125 + 1466)	162
Ca^{2+} (mg)	73.7	2311 (NK + 2311)	NK
Mg^{2+} (mg)	157.4	503 (NK + 503)	NK
total fertilizer applied	7.4 g	81 mL + 10.7 g	81 mL

[a] NK, not known.

Se 0.001%, was applied on days 6, 14, 34, 41, and 48 with the applied dose being 1.48 g/plant at each occasion. Mineral fertilizer was applied on the soil surface around the plants and mixed by tilling the soil.

Urine fertilizer (NPK g/L 8.36−0.7−2) was arranged so that the applications were 16.2 mL/pot on the same cultivation days as above. The urine was applied on the same days as mineral fertilizer, and this was applied with a measuring beaker and sprinkled on the soil around the plants. The soil surface was raked before and after application of urine fertilizer so that the liquid could be better absorbed.

In a similar manner, wood ash (P = 36, K = 137 g/kg) was applied at 3 days after application of the urine fertilizer to avoid possible ammonium evaporation due to pH elevation with ash, and it was applied at a dose of 2.14 g/plant on each occasion. Wood ash was applied around the plants and mixed by tilling the soil.

Urine Collection and Hygiene. The urine for the experiment was collected during the winter 2007−2008 from several eco-toilets in private homes located in the vicinity of Tampere, Finland, and it was stored for about 6 months at 7 °C. The stored urine was analyzed for its microbiological properties. *Salmonella* spp., fecal coliforms, clostridia, enterococci, and coliphages were determined from the mixed urine samples. *Salmonella* spp. was determined by the ISO 6579 method (*13*) including confirmative plate count on XLD and Rambach media (Merck, Darmstadt, Germany). Fecal coliforms, enterococci, clostridia, and coliphages with two *Escherichia coli* hosts, that is, ATCC 13706 and ATCC 15597, were determined according to the standard method used by Pradhan et al. (*6*). For counting geometric means or log-transformed numbers for statistical significances, half of the detection limit (i.e., 0.5 CFU or PFU/mL) was used when the counts in all plates in the same replicate were zero.

The nutrient contents of the urine, total-N, NH_4^+-N, NO_3^--N, NO_2^--N, total-P, PO_4^--P, Cl^-, and K^+, were analyzed according to SFS standard methods, which correspond to APHA methods (*14*). Total-N was analyzed by using the Kjeldahl method (*15*). P and K^+ were determined by colorimetric methods as described in Pradhan et al. (*6*), and NH_4^+-N was determined by spectrophotometry (*16*). NO_3^--N, NO_2^--N, and Cl^- were analyzed by ion chromatography (Dionex DX-120, AS40 automated sampler, Dionex Corp., Sunnyvale, CA).

Wood Ash Collection and Nutrients. Birch tree (*Betula* spp.) wood ash was collected from the furnace of a household in Nilsiä, Finland. The collected ash was sieved and used within 2−3 months. The nutrient contents of the wood ash, total-P, total-K, Ca^{2+}, and Mg^{2+}, were analyzed according to SFS standard methods. Total-P was analyzed by colorimetric method (*17*), and K^+, Ca^{2+}, and Mg^{2+} were analyzed by FAAS (K^+ (*18*); Ca^{2+}, Mg^{2+} (*19*)).

Plant Care, Measurements, and Harvesting. Tomato plants were irrigated with nearby lake water (1 mg of P/L) every day as needed. Flowers were hand pollinated every morning with the help of a soft brush. The vine length, the number of branches in the main vine, and the area of the largest leaf of each plant were measured every seventh day to determine the growth rate of the plants. Fully developed flowers and fruits were counted on every seventh day to determine the impact on the reproductive parts. Harvesting of the tomato fruits started on the 62nd day and ended on the 88th day of cultivation (after seedling transplantation). Fruit biomass was weighed separately, and total biomass of whole plants was weighed as dry weight.

Microbial Analysis. The tomato fruits were analyzed for fecal coliforms, enterococci, clostridia, and coliphages (ATCC 13706 and 15597) with similar methods as used for urine analysis.

Nutrient Analyses. The whole tomato fruits were lyophilized and milled prior to nutrients analysis. NO_3^-, NO_2^-, and Cl^- were determined according to similar methods as used for urine (*6*). The lyophilized and milled tomato powder samples were used for the analysis of protein contents, soluble sugar (sucrose, D-glucose, D-fructose), lycopene, and β-carotene. NO_3^-, NO_2^-, Cl^-, and total-N were determined according to the same standard analysis methods as used for urine.

Soluble Sugar Analyses. Approximately 100 mg of lyophilized tomato powder sample was weighed into a conical flask; 50 mL of ion-free water was added and stirred for 1 h to extract the water-soluble sugars. The solution was filtered, and subsequently the filtrate was treated with Carrez-I and Carrez-II solutions and 0.1 M NaOH to deproteinize the sample, and then it was filtered again to separate the precipitated protein.

This solution was transferred into the cuvettes and monitored with enzymatic reactions at different time intervals for estimated of sucrose, D-glucose, and D-fructose separately. After the inculcations, the samples were determined in a spectrophotometer and the absorbance was recorded at a wavelength of 340 nm (*20*). The kit used for this assay was the enzymatic bioanalysis/food analysis from R-Biopharm, Germany.

Lycopene and β-Carotene Analysis. Approximately 50 mg of lyophilized tomato powder was weighed into the screw-cap tubes, and 0.2 mL of ethanol containing 0.01% BHT (*tert*-butylated hydroxytoluene, Fluka Co., Buchs, Switzerland) and 0.8 mL ion-free water (18.2 MΩ) was added. The solution was extracted twice with 4 mL of hexane (Rathburn Chemicals Ltd., Walkerburn, Scotland) containing 0.01% BHT. The hexane phases were separated by placing the tubes at −70 °C for about 45 min. The extracts were mixed and evaporated under nitrogen flow. The dried extract samples were dissolved in 1 mL of mobile phase (a mixture of acetonitrile/methanol/chloroform 60:25:15, v/v/v, containing 0.01% w/v BHT) and diluted 1:10 with mobile phase prior to the HPLC analysis. This is a modified version of the method used before (*21*).

The HPLC (Shimadzu system, Kyoto, Japan) was equipped with an isocratic solvent delivery pump (LC-10ATvp), a system controller (SCL-Avp), a cooled autosampler equipped with a 50 μL injection loop (SIL-10ADvp), a vacuum membrane degasser (DGU-14A), and a Beckman 168 diode array detector (Beckman). Class WP software (version 6.1) from Shimadzu was used for instrument control, data acquisition, and data processing. Chromatographic separations were performed on a pair of Synergy Hydro-RP 80A (150 mm × 4.6 mm) 4 μm columns coupled in series and a C18 guard column (4 mm × 3 mm) (Phenomenex, Torrance, CA) (*21*).

Stock solutions of β-carotene (0.5 mg/mL), lycopene (0.1 mg/mL), and β-apo-8′-carotenal (1 mg/mL) from Sigma-Aldrich Co., St. Louis, MO (internal standard), were prepared in a solution containing acetonitrile/methanol/chloroform (18:7.5:74.5, v/v/v) and 0.01% (w/v) BHT. Stock solutions were further diluted in the mobile phase containing 0.01% (w/v) BHT to provide working standards with concentrations of 0.18−0.90 μmol/L for carotenoids. The absorbance of working standard solutions was determined by in a Beckman Du 800 spectrophotometer (Beckman Coulter, Fullerton, CA), and the concentration of the working standard was calculated from the specific molar extinction coefficient. The molar extinction coefficients for *all-trans*-lycopene and β-carotene were obtained from Thurnham et al. (*22*). An internal standard was prepared by adding an appropriate amount of β-apo-8′-carotenal dilution to an ethanol/0.01% (w/v) BHT solution. The internal standard was protected from light in an amber glass bottle and stored at −20 °C.

The assay (*21*) was done as 50 μL injection volume with 1 mL/min flow rate (backpressure = 70−80 bar) and a 35 min run time. Lycopene and β-carotene were monitored at 454 nm. The tray compartment containing sample vials was cooled at 4 °C during analysis. The compounds were quantified after a chromatographic separation according to the internal standard method using pure lycopene and β-carotene, and the results were calculated as milligrams per kilogram of fresh weight (FW).

Taste Assessment. Triangle taste testing was done with mineral, urine + ash, and urine fertilized tomatoes due to the limited amount of control tomato fruits, but ordinal taste testing was done for tomatoes from all treatments. These tests were conducted with a panel of 20 individuals; the ability of panel participants to recognize basic tastes (sweet, sour, salty, and bitter) had been pretested (*23*). The taste test was done as described by Pradhan et al. (*6*).

Statistical Analyses. Raw data were stored in MS Excel, and basic parameters were calculated to characterize the overall feature of the data sets. The raw and transformed data were performed for normality test prior to the other statistical analyses. The data from fruit biomass, number of fruits, and microbial and chemical analyses were analyzed with SPSS 14.0 for Windows Statistical Package using General Linear Models (ONEWAY procedure) combined with Tukey's post hoc test. The correlation between parameters was analyzed by Pearson correlation test (with two-tailed test of significance) and Spearman correlation test. The statistical test of the taste test was done by comparing the data with the statistical table (*24*).

RESULTS AND DISCUSSION

Urine Quality. Urine had a strong odor of ammonia due to the high amount of NH_4^+-N present. No enterococci, clostridia, and

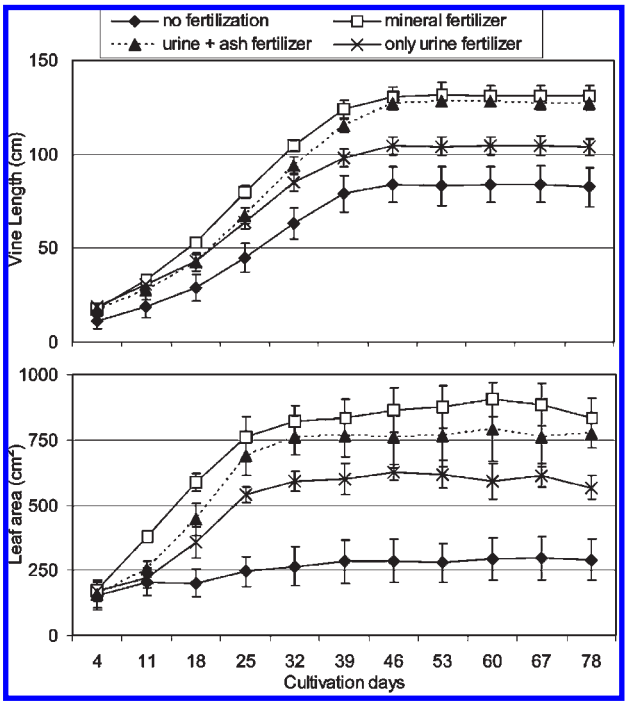

Figure 1. Growth rate of tomato plants from different fertilizer treatments on the basis of leaf area and vine length on different cultivation days (arithmetic mean (AM) and standard deviation (SD) bar) (N = 5). Spearman's analysis showed that the growth rates of vine length and leaf area were positively correlated (r = 0.910, P = 0.0001 and N = 44).

RNA coliphages (with the host *E. coli* ATCC 15597) were detected in the urine sample used (detection limits = 1 CFU/mL or 1 PFU/mL). Similarly, no *Salmonella* spp. (detection limits = 1 CFU/20 mL) and no fecal coliforms (detection limits = 1 CFU/30 mL) were detected in the urine sample used. This might be due to its high pH (> 9), rendering it unsuitable for microbes (*25*). However, a few DNA coliphages (with host *E. coli* ATCC 13706), that is, 96 ± 16 PFU/mL of urine, were detected in the urine sample used. The physical and chemical parameters of the urine are given in **Table 2**.

Growth of the Plants. The fertilizer value of urine (*4*−*8*), (*26*) and wood ash (*2*, *9*−*12*) has been studied previously, but as far as we know our study is the first to use both fertilizers together. The growth of the tomato plants with all treatments was normal except for one plant from the urine + ash treatment, which exhibited growth disturbances from the very beginning; this plant was excluded. The results of this study show that since 25 days after transplantation, the growth rates of the plants (vine length and leaf area) with mineral fertilizer, urine + ash fertilizer, and urine fertilizer were significantly higher (P < 0.05) compared to that obtained without fertilization. On the other hand, at 39 days after transplantation, the growth rates of plants from mineral and urine + ash fertilizer treatments were also significantly higher compared to that obtained with urine alone (**Figure 1**). One possible explanation of this result might be that the urine + ash fertilizer treatment was supplemented better with P, K^+ Ca^{2+}, Mg^{2+}, and other unanalyzed elements. Another possible explanation might be the applied N in urine was used more for fruit protein synthesis as the urine fertilized tomato fruit contained about 35−37% higher protein compared to mineral and urine + ash fertilized tomato fruits. At 46 days the growth had stabilized (**Figure 1**).

Flowering, Fruiting, and Biomass. The first flower bloomed on the 25th day after seedling transplantation in plants from all

Table 4. Yield Components of Tomatoes Obtained from Different Fertilizer Treatments (AM ± SD) ($N = 5$)[a]

measurement	fertilizer treatments				F	P
	none	mineral	urine + ash	urine		
fruit production/plant (g of FW)	91.0 ± 42.7 a	488.8 ± 96.9 b	354.5 ± 34.1 c	385.2 ± 51.0 bc	38.291	0.0001
fruit size (g)	37.1 ± 8.4 a	55.7 ± 2.9 b	53.7 ± 5.0 b	55.4 ± 5.0 b	12.461	0.0001
no. of fruits/plant	2.6 ± 1.4 a	8.6 ± 1.6 c	6.3 ± 0.3 b	6.6 ± 0.8 bc	23.946	0.0001
plant biomass (g of DW)	12.3 ± 3.2 a	45.5 ± 4.4 c	39.7 ± 4.4 c	22.9 ± 1.5 b	86.203	0.0001

[a] The P and F values were obtained from ANOVA analysis for comparison of the variables from different treatments. FW, fresh weight.

Table 5. Chemical Contents in Tomato Fruits per Kilogram of Fresh Weight (AM ± SD) ($N = 5$)[a]

chemical	fertilizer treatments				F	P
	none	mineral	urine + ash	urine		
NO_3^- (mg)	284.6 ± 59.3 b	341.9 ± 42.6 b	328.5 ± 20.0 b	162.8 ± 48.5 a	15.594	0.0001
NO_2^- (mg)	27.4 ± 3.8 a	58.3 ± 10.8 b	52.1 ± 23.0 ab	17.0 ± 14.4 a	8.361	0.002
Cl^- (mg)	401.8 ± 78.1 c	263.1 ± 32.1 ab	537.2 ± 67.0 d	336.3 ± 83.1 bc	14.609	0.0001
Mg^{2+} (mg)	87.9 ± 14.1 a	95.7 ± 3.9 ab	109.9 ± 14.3 b	88.2 ± 7.7 a	4.382	0.020
Ca^{2+} (mg)	36.0 ± 7.1 b	24.3 ± 4.5 ab	22.6 ± 5.3 a	26.2 ± 9.3 ab	3.857	0.030
K^+ (g)	2.9 ± 0.3 c	2.4 ± 0.1 b	2.8 ± 0.1 c	1.2 ± 0.1 a	71.703	0.0001
P (mg)	367.6 ± 43.7 b	396.1 ± 52.4 b	14.7 ± 0.4 a	14.3 ± 2.4 a	161.358	0.0001

[a] The P and F values were obtained from ANOVA analysis for comparison of the variables from different treatments.

treatments, and the first fruit was developed in urine + ash and urine fertilized plants; the result of total number of fruits is presented in **Table 4**. Previous studies (*4*, *6*) described the high yield production in urine fertilized plants compared to mineral fertilized plants, but the N application in urine fertilized plants was much higher in a cucumber study (*4*). Our study showed that the tomato fruit yield in mineral and urine fertilized plants did not significantly differ. However, tomato fruit yield in mineral fertilized plants was significantly higher compared to the urine + ash fertilizer treatment (**Table 4**), and this might be due to loss of N with increasing soil pH because of ash (*27*). On the other hand, tomato fruit production of urine + ash fertilized plants was significantly higher compared to nonfertilized plants, and this finding is in agreement with previous studies (*6*, *8*). However, urine fertilized plants can produce 4.2 times more tomato fruits compared to nonfertilized plants, and it could be even higher if N evaporation could be minimized. There are other ways to interpret this result; urine from a single human individual (500 L/year) could fertilize some 6300 tomato plants, which could produce 2.41 tons of tomato fruits.

Dry biomass of the mineral and urine + ash fertilized plants (above ground) was significantly higher compared to those of other treatments. This high dry biomass in urine + ash fertilizer compared to urine only fertilizer might be due to higher P, K, Ca^{2+}, and Mg^{2+} contents in the ash fertilizer (**Tables 2** and **3**); however, this did not result in higher fruit yield (**Table 4**). The Mg^{2+} content in ash was supposed to be responsible to increase the yield in the crops (*28*). Dry biomass of the urine fertilized plants was higher compared to nonfertilized plants, similar to the result presented by Mnkeni et al. (*8*). Spearman's analysis indicated that the dry biomass of plant (above ground) was positively correlated ($r = 0.905$, $P = 0.0001$, and $N = 20$) with the vine length at 78 days of cultivation.

Microbial Quality of Tomato Fruit. No fecal coliform, enterococci, clostridia, and coliphages were found in any of the tomato fruit samples (detection level = 10 CFU or PFU/g of tomato fruit) as the fruits develop away from the soil surface. However, it is always important to remember that the urine fertilizer should be spread into the soil around the plants and not applied directly to any part of the plants to avoid microbial contamination and possible burning from ammonia. One basic requirement for the urine use in fertilization is that there should not be any fecal cross-contamination occurring during storage of the urine (*29*).

Nutrients Content in Tomato Fruits. This study showed that the NO_3^- and K^+ concentrations were significantly lower in urine fertilized tomato fruits compared to these elements in tomato fruits from other treatments (**Table 5**). In addition, our study showed that the NO_3^- concentration was positively correlated with K^+ ($r = 0.678$, $P = 0.001$, and $N = 20$), and this might be due to cations stimulating nitrate uptake as reported in maize (*30*). On the other hand, P was significantly lower in urine + ash and urine fertilized tomato fruits, and Spearman's analysis revealed a negative correlation ($r = -0.544$, $P = 0.029$, and $N = 16-20$) between P and Cl^-. This could be due to high Cl^- contents in urine, and this is in agreement with the paper of Angelo et al. (*31*). The Mg^{2+} content was significantly higher in urine + ash fertilized tomato fruits compared to urine and no fertilization treatments, perhaps due to the high amount of Mg^{2+} in the ash. Bierman and Rosen (*32*) reported that the Mg^{2+} concentration was greater in ash (sewage sludge ash) fertilized lettuce and corn. This elevation in Mg^{2+} content in vegetables could confer beneficial health properties (*33*). Although Ca^{2+} and K^+ contents were high in ash fertilizer, the amounts of these elements were similar in tomato fruits fertilized with ash or with other fertilizers (**Table 5**). This might be due to the fact that whole Ca^{2+} and K^+ present in ash are not readily bioavailable (*2*).

Protein, Soluble Sugars, and Flavor. The protein content was significantly higher ($P = 0.020-0.0001$) in urine fertilized tomato fruits compared to that in tomato fruits from the other treatments (**Table 6**). This might be due to better availability of N to allow more seeds and protein synthesis. Brennan et al. (*34*) demonstrated that N application could increase the numbers of seeds and the protein content in canola. However, the amount of protein in all tomato fruits was lower than the value presented by Souci (*35*), and this might be due to the fact that the tomato plants were cultivated in small pots with limited root growth. The soluble sugar concentrations in this study (**Table 6**) were slightly greater than in the data presented by Souci (*35*). There were no differences in the taste of tomato fruits from mineral and urine fertilizer treatments, which might be due to their similar soluble sugar contents, but urine and urine + ash fertilizer treatments differed from one another ($P < 0.05$). However, the panelists did

Table 6. Major Primary and Secondary Metabolites Present of Tomato Fruits Obtained with Different Fertilizer Treatments[a]

	fertilizer treatments					
nutrient	none	mineral	urine + ash	urine	*F*	*P*
protein (g)	3.7 ± 0.8 a	4.7 ± 0.5 a	4.6 ± 0.6 a	6.3 ± 1.1 b	9.337	0.001
D-glucose (g)	22.3 ± 1.2 ab	24.2 ± 1.5 b	21.3 ± 0.9 a	23.6 ± 0.6 b	6.934	0.003
sucrose (g)	0.1 ± 0.1	0.8 ± 1.2	0.1 ± 0.1	0.1 ± 0.1	1.122	0.39
D-fructose (g)	25.5 ± 1.4 b	26.8 ± 1.5 b	23.4 ± 1.0 a	25.8 ± 0.7 b	7.335	0.003
lycopene (mg)	5.4 ± 0.9	6.5 ± 2.4	5.1 ± 2.0	5.7 ± 2.6	0.612	0.617
β-carotene (mg)	0.5 ± 0.2 a	0.9 ± 0.2 ab	0.7 ± 0.3 ab	1.1 ± 0.4 b	4.940	0.013

[a] The results are presented per kilogram of FW (AM ± SD) ($N = 5$). Protein was calculated as total-N multiplied by a factor of 6.25. The *P* and *F* values were obtained from ANOVA analysis for comparison of the variables from different treatments.

not prefer any particular samples ($P > 0.05$), and all tomato samples were evaluated as being equally good by the tasters.

Lycopene and β-Carotene. The lycopene content did not differ ($P > 0.05$) in tomato fruits from all treatments (**Table 6**), and it was not influenced by supplementation of P and K^+ (*36*, *37*). However, the lycopene content in our study was lower than reported in the previous studies, that is, 25.7 mg/kg of FW (*38*) and 18.9−25.6 mg/kg of FW (*39*). The low lycopene content in our study might be due to low-intensity light and lower temperature during cultivation (*40*). Surprisingly, the β-carotene level was significantly greater ($P = 0.009$) in tomato fruits from urine fertilizer compared to nonfertilized tomato fruits (**Table 6**). However, this value is less than the value presented by the U.S. Department of Agriculture (*38*), that is, 4.5 mg/kg of FW. The low concentration of β-carotene might be influenced by the temperature and the intensity of light as has been reported for cherry tomato (*41*).

Our result suggests that a weak link may exist between the β-carotene content in tomato fruits and use of fertilizer as reported in kale (*42*) and watercress (*43*). These findings suggest that it is important to use fertilizer in vegetables and fruits to increase the yields and contents of some very important chemical compounds such as the carotenes. β-Carotene content in fruits should be higher from a public health stand point as it is a precursor of vitamin A.

Residual Nutrients in Soil. Total-N amount in the soil after cultivation was elevated in all treatments compared to that in the soil before cultivation (**Table 1**). The possible reason for this result could be the increase of organic matter (OM) in the soil with cultivation. Here, soil nitrogen can be increased by 270−400% compared to bare soils by cultivation with urine and urine + ash fertilization, respectively. The residual NO_3^--N content was lower in the soil from urine + ash fertilizer treatment compared to mineral fertilizer treatment. One possible explanation is that the NO_3^--N and/or NH_4^+-N uptake might have been greater due to the higher pH in urine + ash fertilizer treatments compared to the others. Another possible explanation for lower NO_3^--N could be the high denitrification rate due to application of ash (*27*, *44*). The Cl^- amount in soil fertilized with urine + ash and urine fertilizer was greater due to Cl^- in applied urine, but this could be easily washed out with rainwater. The residual soil conductivity was highest in soil treated with mineral fertilizer, which might be due to high residual NO_3^--N in soil with this treatment as the nitrate content in soil and its electrical conductivity are strongly positively correlated ($r = 0.99$) (*45*).

The level of residual P was greater in urine + ash fertilized soil compared to urine fertilized soil (**Table 1**). This result suggests that the use of urine + ash fertilizer can increase by about 25% residual P in cultivated soil, which is very important for many crops. The level of residual K^+ was greater in urine fertilized soil, which may be due to the fact that plants might take up Na^+ instead of K^+ as previously reported for tomato plants (*46*). This explanation may also account for the lower K^+ contents in tomato fruits of urine fertilized.

In conclusion, although the amounts of applied N were similar with all fertilizer treatments, the tomato yield and aboveground plant dry weight were highest in the mineral fertilizer treatment, most likely because the number of fruits per plant was highest (**Table 4**). However, the present results are important in at least two respects: (1) The fruit yields of mineral fertilizer and urine only treatment were not significantly different, and the treatment urine + ash could also produce a >3-fold improvement in fruit yield compared to no fertilization. (2) The microbial and principal chemical compounds including antioxidants in tomato fruits from urine + ash and urine fertilized plants were similar to those of fruit obtained after mineral fertilization. Our results confirm most of the study hypotheses, emphasizing the fertilizer value of urine and wood ash. Findings from this study have important implications. They may contribute to the development of positive attitudes about the use of urine and ash as fertilizer as a way to both increase crop yield and reduce water pollution. In addition, findings from this study may contribute to optimize the use of urine and wood ash to fertilize crops, which will help to maintain fruit yield quality, soil potential, and to control N emission. More research on this topic needs to be undertaken before the response of the different elements present in urine and ash is more clearly understood so that this can be used in the cultivation of different food plants.

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LITERATURE CITED

(1) Schouw, N. L.; Danteravanich, S.; Mosbaeck, H.; Tjell, J. C. Composition of human excreta, a case study from southern Thailand. *Sci. Total Environ.* **2002**, *286*, 155–166.

(2) Erich, M. S. Agronomic effectiveness of wood ash as a source of phosphorus and potassium. *J. Environ. Qual.* **1991**, *20*, 576–581.

(3) Huotari, N.; Tillman-Sutela, E.; Pasanen, J.; Kubin, E. Ash-fertilization improves germination and early establishment of birch (*Betula pubescens* Ehrh.) seedlings on a cut-away peatland. *For. Ecol. Manage.* **2008**, *255*, 2870–2875.

(4) Heinonen-Tanski, H.; Sjöblom, A.; Fabritius, H.; Karinen, P. Pure human urine is a good fertilizer for cucumbers. *Bioresour. Technol.* **2007**, *98*, 214–217.

(5) Guzha, E.; Nhapi, I.; Rockstrom, J. An assessment of the effect of human faeces and urine on maize production and water productivity. *Phys. Chem. Earth* **2005**, *30*, 840–845.

(6) Pradhan, S. K.; Nerg, A.-M.; Sjöblom, A.; Holopainen, J. K.; Heinonen-Tanski, H. Use of human urine fertilizer in cultivation of cabbage (*Brassica oleracea*)—impacts on chemical, microbial and flavor quality. *J. Agric. Food Chem.* **2007**, *55*, 8657–8662.

(7) Tidåker, P.; Mattsson, B.; Jönsson, H. Environmental impact of wheat production using human urine and mineral fertilizers—a scenario study. *J. Cleaner Prod.* **2007**, *15*, 52–62.
(8) Mnkeni, P. N. S.; Kutu, F. R.; Muchaonyerwa, P. Evaluation of human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in the Eastern Cape, South Africa. *Waste Manage. Res.* **2008**, *26*, 132–139.
(9) Meyers, N. L.; Kopecky, M. J. Industrial wood ash as a soil amendment for crop production. *TAPPI J.* **1998**, *81*, 123–130.
(10) Patterson, S. J.; Acharya, S. N.; Thomas, J. E.; Bertschi, A. B.; Rothwell, R. L. Barley biomass and grain yield and canola seed yield response to land application of wood ash. *Agron. J.* **2004**, *96*, 971–977.
(11) Merino, A.; Otero, V.; Omil, B.; Lastra, B.; Pineiro, V.; Gallego, P. P. Application of wood ash compared with fertigation for improving the nutritional status and fruit production of kiwi vines. *J. Plant Nutr. Soil Sci.* **2006**, *169*, 127–133.
(12) Moyin-Jesu, E. I. Use of plant residues for improving soil fertility, pod nutrients, root growth and pod weight of okra (*Abelmoschus esculentum* L). *Bioresour. Technol.* **2007**, *98*, 2057–2064.
(13) ISO 6579. General guidance on methods for the detection of *Salmonella*, **1993**.
(14) American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; Washington DC, 2005.
(15) ISO 1871. Agricultural food products—general directions for the determination of nitrogen by the Kjeldahl method, **1975**.
(16) SFS-EN ISO 11732. Water quality. Determination of ammonium nitrogen. Method by flow analysis (CFA and FIA) and spectrometric detection, **2005**.
(17) SFS-EN 13346. Characterization of sludges. Determination of trace elements and phosphorus. Aqua regia extraction methods, **2000**.
(18) SFS 3017. Metal content of water determined by atomic absorption spectroscopy, atomization in flame. Special guidelines for sodium and potassium, **1982**.
(19) SFS 3018. Metal content of water determined by atomic absorption spectroscopy, atomization in flame. Special guidelines for calcium and magnesium, **1982**.
(20) Sekin, S. Enzymatic determination of glucose, sucrose and fructose in tobacco. *Tobacco Sci. 23*, 75−77; *Tobacco Int.* **1978**, *181*, 27−29.
(21) Karppi, J.; Nurmi, T.; Olmedilla-Alonso, B.; Granado-Lorenciob, F.; Nyyssönen, K. Simultaneous measurement of retinol, α-tocopherol and six carotenoids in human plasma by using an isocratic reversed-phase HPLC method. *J. Chromatogr., B.* **2008**, *867*, 226–232.
(22) Thurnham, D. I.; Smith, E.; Flora, P. S. Concurrent liquid-chromatographic assay of retinol, α-tocopherol, *β*-carotene, α-carotene, lycopene, and *β*-cryptoxanthinin in plasma, with tocopherol acetate as internal standard. *Clin. Chem.* **1988**, *34*, 377–381.
(23) Meilgaard, M.; Civille, G. V.; Carr, B. C. *Sensory Evaluation Techniques*, 3rd ed.; CRC Press: Boca Raton, FL, 1991; pp 61−68.
(24) Roessler, E. B.; Pangborn, R. M.; Sidel, J. L.; Stone, H. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle test. *J. Food Sci.* **1978**, *43*, 940–947.
(25) Chandran, A.; Pradhan, S. K.; Heinonen-Tanski, H. Survival of enteric bacteria and coliphage MS2 in pure human urine. *J. Appl. Microbiol.* **2009**, in press, doi:10.1111/j.1365-2672.2009.04353.x.
(26) Kirchmann, H.; Pettersson, S. Human urine—chemical-composition and fertilizer use efficiency. *Fert. Res.* **1995**, *40*, 149–154.
(27) Martikainen, P. J. Nitrification in forest soil of different pH as affected by urea, ammonium sulphate and potassium sulphate. *Soil Biol. Biochem* **1985**, *17*, 363–367.
(28) Oliveira, I. P.; Asher, C. J.; Edwards, D. G.; Santos, R. S. M. Magnesium sulphate and the development of the common bean cultivated in an Ultisol of northeast Australia. *Sci. Agric.* **2000**, *57*, 153–157.
(29) Höglund, C.; Ashbolt, N.; Stenström, T. A.; Svensson, L. Viral persistence in source-separated human urine. *Adv. Environ. Res.* **2002**, *6*, 265–275.
(30) Ivashikina, N. V.; Feyziev, Y. M. Regulation of nitrate uptake in maize seedlings by accompanying cations. *Plant Sci.* **1998**, *131*, 25–34.
(31) Angelo, S.; Pietro, S.; Francesco, S. Influence of salinity source on production, quality and environmental impact of tomato grown in a soilless closed system. *J. Food Agric. Environ.* **2008**, *6*, 357–361.
(32) Bierman, P. M.; Rosen, C. N. Sewage sludge incinerator ash effects on soil chemical-properties and growth of lettuce and corn. *Soil Sci. Plant Anal.* **1994**, *25*, 2409–2437.
(33) Larsson, S. C.; Wolk, A. Magnesium intake and risk of type 2 diabetes: a meta-analysis. *J. Intern. Med.* **2007**, *262*, 208–214.
(34) Brennan, R. F.; Bolland, M. D. A. Effect of fertilizer phosphorus and nitrogen on the concentrations of oil and protein in grain and the grain yield of canola (*Brassica napus* L.) grown in south-western Australia. *Aust. J. Exp. Agric.* **2007**, *47*, 984–991.
(35) Souci, S. W.; Fachman, W.; Kraut, H. *Food Composition and Nutrition Tables*, 5th revised and completed ed.; Medpharm Scientific Publishers: Stuttgart, Germany, 1994; pp 894−895.
(36) Oke, M.; Ahn, T.; Schofield, A.; Paliyath, G. Effects of phosphorus fertilizer supplementation on processing quality and functional food ingredients in tomato. *J. Agric. Food Chem.* **2005**, *53*, 1531–1538.
(37) Fontes, P. C. R.; Sampaio, R. A.; Finger, F. L. Fruit size, mineral composition and quality of trickle-irrigated tomatoes as affected by potassium rates. *Persquisa Agropecuaria Brasileira* **2000**, *35*, 21–25.
(38) USDA, Agricultural Research Service, Nutrient Data Laboratory. *National Nutrient Database for Standard Reference*, http://www.nal.usda.gov/fnic/foodcomp/search/, **2008**.
(39) Hernández, M.; Rodríguez, E.; Díaz, C. Free hydroxycinnamic acids, lycopene, and color parameters in tomato cultivars. *J. Agric. Food Chem.* **2007**, *55*, 8604–8615.
(40) Heinonen, M. I.; Ollilainen, V.; Linkola, E. K.; Varo, P. T.; Koivistoinen, P. E. Carotenoids in Finnish foods: vegetables, fruits and berries. *J. Agric. Food Chem.* **1989**, *37*, 655–659.
(41) Zanfini, A.; Dreassi, E.; La Rosa, C.; D'Addario, C.; Corti, P. Quantitative variations of the main carotenoids in Italian tomatoes in relation to geographic location, harvest time, varieties and ripening stage. *Ital. J. Food Sci.* **2007**, *19*, 181–190.
(42) Kopsell, D. A.; Kopsell, D. E.; Curran-Celentano, J. Carotenoid pigments in kale are influenced by nitrogen concentration and form. *J. Sci. Food Agric.* **2007**, *87*, 900–907.
(43) Kopsell, D. A.; Casey Barickman, T. C.; Sams, C. E.; Scott McElroy, J. Influence of nitrogen and sulfur on biomass production and carotenoid and glucosinolate concentrations in watercress (*Nasturtium officinale* R. Br.). *J. Agric. Food Chem.* **2007**, *55*, 10628–10634.
(44) Maljanen, M.; Jokinen, H.; Saari, A.; Strömmer, R.; Martikainen, P. J. Methane and nitrous oxide fluxes, and carbon dioxide production in boreal forest soil fertilized with wood ash and nitrogen. *Soil Use Manage.* **2006**, *22*, 151–157.
(45) Stamatiadis, S.; Werner, M.; Buchanan, M. Field assessment of soil quality as affected by compost and fertilizer application in a broccoli field (San Benito County, California). *Appl. Soil Ecol.* **1999**, *12*, 217–225.
(46) Tuna, A. L.; Kaya, C.; Ashraf, M.; Altunlu, H.; Yokas, I.; Yagmur, B. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. *Environ. Exp. Bot.* **2007**, *59*, 173–178.

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